REMARKS

Claims 14, 23-25 and 32-37 are pending in the subject application. Applicants have canceled claim 32.

REJECTIONS MAINTAINED UNDER 35 U.S.C. 103 (a)

Claims 23 and 25 drawn to single chain antibodies that are free end specific for amyloid beta peptides stand rejected by the Examiner under 35 U.S.C 103 (a) as being allegedly unpatentable over anyone of Konig et al., (Ann NY Acad. Sci 777:345-355, 1996) or in view of Seubert et al. (US Patent 6,114,133), issued September 5, 2000 and filed November 14, 1994 and Duenas et al.

Applicant traverses the Examiner's rejections of the claims. Contrary to the Examiner's unsupported assertions Applicant contends that there is no evidence that the monoclonal antibody described in Konig does not bind the intact APP protein. Specifically, in Konig, no labeling was seen with either antibody (286.8A or 369.2B) used in this study. There is no indication or evidence provided by Konig et al. that the N-terminal antibody (286.8A), should not bind the precursor protein which is present in all cells. In fact, the 286.8A antibody was generated against a crude synthetic Aβ 1-42 preparation and was shown previously to be specific for amino acids 3-8 (see page 350). Antibody 286.8A is therefore **NOT** free-end specific and would be expected to bind to the precursor protein. The negative labeling results pointed out by the Examiner holds true for <u>both</u> antibodies tested would therefore lead one skilled in the art to believe that the experimental conditions used were not appropriate for intracellular staining by either of these antibodies. An increasing number of antigens once considered undetectable in tissue staining are in fact found to be reliably detected when one uses the appropriate 'antigen recovery' technique.

In addition, several studies have recently shown that human AD brain tissue stained with antibodies specific to the C-terminus of Aβ 1-42 reveal significant amounts of intraneuronal immunoreactivity (D'Andrea, M.R. et al. (2002), Neurosci Lett 333:163-166; Gouras, G. et al. (2000), Am J Pathol 156:5-20; and Mochizuki, A. et al. (2000), The Lancet 355:42-43. This intraneuronal Aβ 1-42 immunoreactivity has been replicated by several different antibodies. The reactivity is specific since it is not detected when staining with preimmune serum or after competition with the Aβ 1-42 peptide. One would expect this same type of intracellular staining with the 369.2B antibody. The fact that no intracellular staining was detected with

antibody 369.2B would lead one skilled in the art to conclude that the conditions used in the Konig study were ineffective. Accordingly, Applicant does not agree with the Examiner's assertion that intracellular staining with antibody 369.2B would demonstrates it binds to the amyloid precursor protein. In fact, one skilled in the art would not appreciate that the monoclonal antibodies did not bind the amyloid β precursor according to the teachings of Konig et al.

In view of the above, Applicant maintains that there is no evidence that the monoclonal antibody described in Konig does not bind the intact APP protein and hence request the Examiner to withdraw the rejection under 103 (a).

NEW REJECTIONS BASED ON THE AMENDMENT
UNDER 35 USC 103(a)

The Examiner rejected claims 14 and 32, which are directed to monoclonal antibody that is free end specific for the free N terminus of an amyloid beta peptide, under 35 USC 103(a) as being allegedly unpatentable over Saido et al., in view of Takeda et al., and Goding.

In addition, the Examiner asserted that Claims 23, 24 and 35 drawn to single chain antibodies are rejected under 35 USC 103 (a) over Saido et al, Takeda et al., and Goding as applied to claims 14 and 32 above and further in view of Seubert et al., and Duenas et al.

In addition, the Examiner rejected claims 33 and 34 drawn to monoclonal antibody that is free end specific for the free C-terminus of an amyloid beta peptide (1-40) or (1-43), which antibody does not bind to the amyloid beta precursor protein from which said amyloid beta peptide may be proteolytically derived, under 35 USC 103 (a) as being allegedly unpatentable over Saido et al., in view of Takeda et al., Seubert et al and Goding.

In addition, the Examiner rejected claims 23, 25, 36 and 37 drawn to single chain antibodies that are free end C-terminal end specific for amyloid beta peptide, which antibody does not bind to the amyloid beta precursor protein from which said amyloid beta peptide may be proteolytically derived, and further limited the claims to amyloid beta (1-40) or (1-43) in claims 36 and 37 under 35 USC 103 (a) as being allegedly unpatentable over Saido et al., in view of Takeda et al., Seubert et al and Goding.

Applicant traverses the Examiner rejections of claims 14, 23, 24, 25, 33, 34, 36 and 37 under 103(a) in view of Saido and in combination with different of the references mentioned above. Applicants contends that a prima facie case of obviousness has not been established.

The combination of Saido with any of the references mentioned above Takeda et al., does not teach or suggest all the limitations of claims 14, 23, 24, 25, 33, 34, 36 and 37 under 103(a). Saido's Reference as discussed below does not teach end-specific **monoclonal** antibodies. All other references are also silent as to Applicant's claimed invention and therefore cannot cure the deficiencies of Saido et al. Accordingly, Applicants respectfully assert that this rejection should be withdrawn.

Specifically, Saido reference is directed to **polyclonal antibodies** made in **rabbits** and is not directed to monoclonal antibodies. A polyclonal response must be identified in an animal before production of monoclonal antibodies is initiated. It is important to note, however, that mice are almost always the animal of choice for monoclonal antibody production, primarily due to the availability and technological success of mouse myeloma cell lines as fusion partners. Available protocols for monoclonal antibody generation strongly advise that an animal with a very high antibody titer be selected for fusion. Moreover, before proceeding with the fusion it is critical that the animal exhibits antibodies of the desired specificity. In an experiment carried out by the Applicants ninety-seven mice were immunized with antigen (DAEFR-C-KLH) in Freund's adjuvant according to standard immunization protocols that should elicit a strong antibody response. Antisera were evaluated for anti Aβ 1-40 antibodies. Significant antibody titer was detectable in only 14/97, representing a 14% response rate (see Table 1). Of these 14 responders, most had either a low or moderate response, whereas only one mouse responded with a high titer. This in is sharp contrast to the response in rabbits. In the same experiment only 2 rabbits were immunized with the same antigen and both demonstrated antibodies to the Aβ 1-40 peptide. This represents a 100% response rate. Furthermore, both can be classified as high responders.

The antisera from low, medium, and high responding mice and from the high-responding rabbits were tested for free-end specificity in the ELISA spanning assay as defined in the Specification. The reactivity of most mouse antisera with the Aβ peptide was significantly inhibited with the spanning peptide (sequence EISEVKMDAEFRHD) and was therefore NOT free-end specific. Those antisera which were inhibited less by the spanning

peptide were not from high responding mice. On the other hand, both rabbits exhibited exquisite end-specificity since the reactivity of their antisera with the Aβ peptide was not inhibited with the spanning peptide, even at the higher concentration used (25 μg/ml).

In an attempt to increase titers, many additional injections were carried out over a period of several months. At final testing, another three animals met the response criteria as described in Table 1 and were classified as medium-responders. Sera of one mouse was inhibited 100% with 10 μg/ml Abeta 1-40 and 16% with the spanning peptide and was therefore chosen for fusion and generation of monoclonal antibodies. The ELISA assay used to identify positive clones from the fusion of the mouse spleen involved reacting hybridoma supernatants at increasing dilutions with Abeta 1-40 or with amyloid precursor protein (APP)-coated plates. Over 500 clones were screened in this process. Seven clones were identified that reacted significantly with the Abeta 1-40 peptide, but not with APP, indicative of their free-end specificity.

Applicant's experiment support the fact that based on the findings of Saido et al., it was not obvious to generate a strong immune response of the desired specificity in the animal of choice for monoclonal antibody production. In fact, an unexpected amount of experimentation was required to identify suitable animals to proceed with. Accordingly, it would not be prima facie obvious to combine the teachings of Saido with any of the mentioned references, to obtain monoclonal-end specific antibodies.

Lastly, in regard to the rejections of claims 25, 33-34 and 36-37, which are directed to monoclonal or single chain antibody, which is free end specific to the free end C terminus of an amyloid beta, in view of Saido in combination with other references mentioned above, Applicant points out that there are structural and physico-chemical differences between antibodies directed to the N-terminus epitopes disclosed in Saido and the C terminus antibodies claimed in claims 25, 33-34 and 36-37. Conformationally, the N-terminal sequences are clearly quite different from the more hydrophobic C-terminal sequences (Chaney, MO et al. Protein Eng. 1998 Sep;11(9):761-7). The N-terminal region of the amyloid beta peptide is also documented to be more immunogenic than other regions of the molecule (Dickey CA et al. DNA Cell Biol. 2001 Nov;20(11):723-9). In fact, the C-terminal ends themselves should each be regarded as a different epitope in terms of structural and chemical properties, and most

importantly, immunogenicity. For example, Aβ 1-40 exhibits among other things a dramatic difference in its solubility in aqueous solutions when compared to Aβ 1-42 (Burdick et al. 1992 JBC 267, 546-554). The latter is virtually insoluble in water, whereas 1-40 is water soluble up to several mg/ml in vitro. Aβ 1-42 is known to be more hydrophobic and more prone to aggregate than Aβ 1-40. Again, this will have a direct affect on sequence accessibility and immunogenicity. Thus, the assertion that 'similar approaches for producing the proteolytic product specific antibodies will be applicable to resolving the differential carboxyl-terminal processing of Aβ peptides' is not accurate and it is not prima facie obvious to combine the teachings the free N-terminal specific antibodies of Saido with any of the other mentioned references, to obtain monoclonal- free C-terminal specific antibodies of claims 25, 33-34 and 36-37. Therefore, Applicant respectfully requests the Examiner to withdraw the rejections of the claims under 103(a).

Should the Examiner have any question or comment as to the form, content or entry of this Amendment, the Examiner is requested to contact the undersigned at the telephone number below. Similarly, if there are any further issues yet to be resolved to advance the prosecution of this application to issue, the Examiner is requested to telephone the undersigned counsel.

Please charge any fees associated with this paper to deposit account No. 05-0649.

Respectfully Submitted,

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Date: October 22, 2003

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